3 studies and 1 case report from the literature published after 1988 were submitted. One of the 3 studies represents a post-marketing trial using Cernevit.

A summary of these submitted studies follows:

1. Study ID: R3: Title: Home Parenteral Nutrition in Children: Bioavailability of Vitamins in Binary Mixtures Preserved For 8 Days:

Investigator: Drs. Ben Hariz, Ricour, et al Study site: France Date of study: 12/90-3/91

The investigator states that Cernevit has been shown to be stable in vitro when mixed with parenteral nutrition mixtures and stored for 8 days. He states the aim of the present study was to compare the plasma vitamin levels, over a 3 month period, in children treated with Cernevit infused with TPN (the binary portion- i.e. glucose, amino acids, electrolytes and micronutrient portion) prepared twice weekly to levels in the same children and the same TPN, but prepared once weekly and stored at 4 degrees C, protected from light.

(Note: the Cernevit used in this study differs from the currently marketed formulation in that the former contains different amounts of vitamin D, B12 and biotin. The Cernevit used in this study contains the following amounts of these vitamins- vitamin D: 220 IU, B12: 0.006 mg and biotin 0.069 mg while in the currently marketed preparation, the respective quantities are: 200 IU, 0.0055 mg and 0.06 mg).

Of the 20 children who were enrolled, 19 were analyzed (6F and 13M), ages: 5 mos.-11 yrs. (note: one patient was 11 yrs., the remaining 18 patients were < 6 yrs. with 6 of these 18 patients being <2 yrs.) 1 of 20 children dropped-out due to technical problems in the dosage of the vitamins. All children had received prolonged home nutrition for a mean of 42 mos. with range of 4 mos.-11 yrs. Lipid emulsions were infused apart from the binary mixture.

For all children, the addition of Cernevit was started at least 1 month before onset of the study. Children <2 yrs. received ½ vial/day (n= 6 patients) and those 2-11 yrs. (n= 13 patients), received 1 vial/day. In addition, the lipid emulsion was supplemented with vitamin E at a dose of 0.6 mg/g of lipids in 17 of the 19 evaluable patients. Therefore, patients received vitamin E in Cernevit and in Intralipid. The total vitamin E intake ranged from 4.3-38.2 mg/day in these 19 patients (~61% - 5.5x recommended by the AMA for children <11 yrs. old receiving TPN).

Water and lipid soluble vitamins were measured at baseline, day 30 and day 90 with the following exceptions: 1,25-OH2 D3 was measured in only 7 children and water soluble vitamins were only analyzed in children > 3 yrs. (n= 12). Blood sampling was performed 6-8 hrs. after nocturnal infusion of the parenteral nutrition by puncturing a peripheral vein. Vitamins A and E were measured by HPLC and, the D vitamins, by comparative protein binding analysis. B1, B2 and B6 were measured by HPLC. Biotin was measured by microbiological assay. Vitamin C was determined by spectrometric measurement of both ascorbic acid and dehyroascorbic acid using nitrophenylenedramine.

Results:

Neither allergic reactions nor intolerance was observed in the course of the study.

No patient developed clinical signs of hypo- or hypervitaminosis.

Mean Blood Levels of the Lipid-Soluble Vitamins at Baseline, Day 30 and Day 90 (NS= not significant):

| Vitamin | Baseline | | Day 30 | | Day 90 | | P value | Reference value |
|---|---|---|---|---|---|---|---|---|
| | N | Mean±SD | N | Mean±SD | N | Mean±SD | | |
| 25-OH D | 18 | 14.4±0.8 | 19 | 14.1±0.7 | 19 | 16.0±0.9 | NS | 9-34 mg/ml |
| 1, 25-OH D | 7 | 38.6±9.9 | 7 | 50.1±9.0 | 7 | 63.6±14.2 | NS | 20-80 mg/ml |
| Vitamin A | 19 | 387.5±30.5 | 19 | 405.8±31.1 | 19 | 368.8±25.6 | NS | 200-800 ug/l |
| Vitamin E alpha | 19 | 8.8±0.8 | 19 | 10.5±0.9 | 19 | 10.8±0.9 | 0.05 | 7-16 mg/l |
| Vit. E gamma | 19 | 0.8±0.2 | 19 | 1.0±0.2 | 19 | 1.0±0.2 | NS | |

Comments:

Vitamin D 25 (OH) levels were normal at study end in all 19 patients, including 1 patient with a low baseline. (Note: 1 patient had a transiently low level on day 30).

Vitamin D 1,25 (OH) levels were normal at study end in 6/7 patients, including 2 patients who had low baseline levels. In 1 patient with a normal baseline value, the vitamin D 1,25 (OH) level became elevated on days 30 and 90 (95 and 146 pg/ml with normal to 80 pg/ml). (Note: only 2 of these 7 patients were <2 yrs. old. Therefore, we have data on the performance of ½ vial Cernevit/day in only 2 patients with respect to 1, 25-OH vitamin D).

In one patient, age 1.6 yrs., with hypergammaglobulinemia and hepatic disease, the vitamin A levels were consistently low, and, in another patient, it was temporarily low (day 30 only). Therefore, in 18/19 patients, the vitamin A levels were normal at baseline and study end (day 90).

Vitamin E alpha (alpha-tocopherol) showed a significant increase during the study, but remained within normal limits. Vitamin E alpha levels normalized in 2/5 patients who had low baseline values. It fell from normal to slightly below normal (6.98 ng/l, with normal of 7-16 ng/l) at study end in 1 patient and remained high throughout the study in another. In summary, vitamin E levels were normal at study end (day 90) in 12/19 patients (10 with normal baseline and 2 with low baseline. Note: the vitamin E level was transiently low- at day 30- in 1 of these 10 patients with normal baseline). Vitamin E levels were high at study end in 3/19 patients (2 of whom had normal baseline but levels of 17.9-19.9 ng/l on day 90) and low in 4 (one of whom had normal baseline).

Mean Blood Levels of the Water-Soluble Vitamins at Baseline, Day 30 and Day 90 (NS= not significant):

| Vitamin | Baseline | | Day 30 | | Day 90 | | P value | Reference value |
|---|---|---|---|---|---|---|---|---|
| | N | Mean±SD | N | Mean±SD | N | Mean±SD | | |
| Biotin | 11 | 529±73 | 11 | 657±109 | 11 | 617±77 | NS | >340 mg/l |
| B1 | 12 | 99 ±3.7 | 12 | 98±4.5 | 12 | 99±3.9 | NS | >79 IU/l |
| B2 | 11 | 1157±52 | 11 | 1189±40 | 11 | 1135±32 | NS | >912 IU/l |
| B6 | 11 | 604±21 | 11 | 606±21 | 11 | 575±30 | NS | >312 IU/l |
| C | 12 | 5.9±0.7 | 12 | 6.1±0.9 | 12 | 6.2±0.8 | NS | >6.2 mg/l |

Comments:

There were no significant changes in the mean concentration of any of the water soluble vitamins from baseline to end of study. Mean vitamin C levels were slightly below normal at baseline and throughout the study. Vitamin C levels normalized by study end (day 90) in only 2/6 patients with low baseline values. It fell from a normal baseline to low throughout the study in 2 patients and was temporarily low- at day 30- in 1 patient with a normal baseline value. In summary, vitamin C levels were normal at study end (day 90) in 6/12 patients (4 of whom had a normal baseline) and low in 6 or 50% (two of whom had a normal baseline level). The investigator notes that vitamin C is unstable in binary mixtures. Although the degradation of vitamin C is equivalent to only 30% after 8 days of preservation at 4 degrees C, this process accelerates at room temperature during the infusion of the bag contents.

Biotin levels were normal at study end in 8/11 patients, including 1 patient who had a low baseline value (note: biotin was transiently low- day 30- in 1 of these 8 patients). However, 3 patients (27%) with normal baseline biotin, had low levels at study end (160, 290 and 300 mg/l).

One patient had a low baseline B1 level which remained low throughout the study. In the remaining patients, the normal baseline level was maintained throughout the study.

Both B2 and B6 levels were normal at baseline in all patients and remained normal throughout the study.

The authors concluded that their results suggest that nutritional bag preparation intervals may be increased from 4 days to 8 days, allowing delivery every 8 days, provided plasma vitamin levels, especially vitamin C, are regularly monitored, e.g. q 3months.

2. Blood Levels of Water-Soluble Vitamins in Pediatric Patients on Total Parenteral Nutrition Using a Multiple Vitamin Preparation
Mariner et al J of Parenteral and Enteral Nutrition 13(2): 176-184, 1989

The purpose of this open, prospective study was to determine blood levels of the water-soluble vitamins in children on TPN receiving a new water soluble vitamin solution, Soluvit N (KabiVitrum Laboratories).

The patients were divided into 2 groups:

Group A: followed for vitamins B1, B2 and B12

Group B: followed for folate, niacin and biotin

Group A consisted of 13 patients, with mean age of 7.5 yrs. and range of 9 mos.-15 yrs. 12/13 patients had been on TPN with vitamins for ≥ 1 year. One patient was on TPN for only 1.5 mos. The TPN had been supplemented with vitamins prior to this study. Most of the vitamins given prior to this study were similar or slightly lower than the vitamins administered in this study. Blood was drawn for vitamin analysis prior to study and, generally on study days 50 (at ~ 8 weeks) and 115 (at ~ 16 weeks).

Group B consisted of 17 infants and children, with mean age of 3.73 years and range of 1 week to 15 years old. Only 9 of these 15 patients received TPN prior to the study. Those who did receive TPN prior to the study, also received multivitamins in their TPN. Most of the vitamins given prior to this study were similar or slightly lower than the vitamins administered in this study. Blood was drawn for vitamin analysis prior to the study and at study days 15 and 30. Duration of follow-up was up to 3 mos. with mean of 2 mos.

Vitamin composition, dosage and administration:

The vitamin composition/vial of Soluvit N, the multivitamin preparation used in this study, was- B1: 3 mg, B2: 3.6 mg, Niacin: 40 mg, B6: 4 mg, Pantothenate: 15 mg, Ascorbic acid: 100 mg, Biotin: 60 mcg, Folic acid: 400mcg and B12: 5 mcg. (Soluvit N contains the same amounts of these water soluble vitamins as MVI-12. However, of these, only biotin is present in the same amount in Cernevit as it is in Soluvit N. Cernevit contains the following quantities of the remaining vitamins as follows: B1- 3.51 mg, B2- 4.14 mg, Niacin- 46 mg, B6- 4.53 mg, Pantothenate- 17.25 mg, Ascorbic acid- 125 mg, Folic acid- 414 mcg and B12- 5.5 mcg).

Patients <18 mos. old, received ½ vial Soluvit N/day. Patients >18 mos. but <10 yrs., received 1 vial/day and patients >10 yrs. received 1.5 vials/day.

In group A, the vitamins were added to the TPN daily, just prior to the start of the infusion.

In group B, the vitamin solution was mixed with the TPN during the TPN mixture preparation which was subsequently filtered.

In both groups, the daily vitamin dosage was diluted in the 24 hr. volume of the TPN solution.

Results:

There were no substantial changes in either hematology or hepatic function during the study.

Group A Vitamin levels:

Mean B1, B2 and B12 levels were above normal at baseline and remained so at the 115 day study timepoint:

| Vitamin | Reference Range | Day 0 | Day 115 |
|---|---|---|---|
| B1 (nmol/l) | 100-200 | 336 + 40 | 263 + 37 |
| B 2 (nmol/l) | 200-400 | 516 + 71 | 437 + 57 |
| B12 (pg/ml) | 250-900 | 1715 + 82 | 1280 + 96 |

Note: since only mean vitamin levels were provided, we do not know if individual patients had low levels of any of these vitamins.

Group B Vitamin levels:

Mean biotin, niacin and folate levels were low normal at baseline and, with the exception, of biotin, they significantly and progressively increased during the study. Mean serum biotin was low normal at baseline (2.2 $\pm$ 0.2 nM; normal: 1.96-2.84 nM), and, after a significant ($p < 0.05$) rise at study day 30 (3.6 $\pm$ 0.4 nM) compared to baseline, the mean levels 60 and 90 days (2.6 $\pm$ 0.4 nM and 2.8 $\pm$ 0.4 nM, respectively) were similar to those at baseline.

Mean whole blood niacin levels were low normal at baseline in patients <10 yrs. of age (36 $\pm$ 2 uM) and they significantly rose throughout the study in this group with the mean level at day 90 (68 $\pm$ 5 uM), exceeding the upper limit of normal (60 uM). Mean niacin levels in patients >10 yrs. of age were at the upper limits of the normal range at baseline (59 uM) and they progressively rose to 72 uM by day 90.

Mean plasma folate levels were low normal at baseline across all age groups and they progressively rose during the study, with mean values at day 90 being 2- to 3-fold higher than baseline. (Note: reference range for plasma folate was specified as >5 ng/ml; at study endpoint, levels were 21-22 ng/ml). Rbc folate levels followed a similar pattern to plasma folate. (Note: it is stated that 40% of the children had low plasma and rbc folate levels at baseline, but it is not specified in how many of these patients, folate levels normalized during the course of the study).

No adverse effects were observed.

The authors concluded that the elevated values for some of the vitamins measured, suggests the need for more studies to define a more precise range of supplementation in different pediatric age groups.

3. Serum Vitamin A and E Concentrations in Pediatric Total Parenteral Nutrition Patients
Hack et al J of Parenteral and Enteral Nutrition 14(2):189-194, 1990

The purpose of this study was to assess the efficacy of LyphoMed Multi Vitamin Concentrate in meeting vitamin A and E requirements in children on TPN.

29 children (17 M and 12 F), ages 1 month- 11 yrs., mean age of 45 mos., on TPN for a mean of 21 weeks (range: 2 weeks- ~ 1.7 years) were enrolled. Vitamin A, 5000 IU, as retinol, and vitamin E, 2.5 IU, as alpha-tocopherol acetate, were added daily to the glucose-amino acid portion of the TPN. (Note: Cernevit contains 3500 IU vitamin A and 11.2 IU vitamin E. The AMA NAG recommendations for full-term infants to children 11 yrs. of age receiving TPN are 2300 IU vitamin A and 7 IU vitamin E). The TPN solutions were kept refrigerated and used within 48 hrs. Intralipid (10% or 20%), if given, was administered in a separate bottle, co-administered via a Y connector distal to the filter on the aqueous TPN solution tubing. This group of patients was compared to a control group of 52 children (35 M and 17 F), ages 1 month- 11 yrs., not on TPN but who were admitted to the hospital for short-admission surgery.

Blood was collected from TPN patients at intervals after study entry (2, 4 and 8 weeks). In 23/29 patients, vitamins A and E were determined on more than 1 occasion. The samples from the control patients were excess serum from blood samples obtained during preoperative screening.

Serum vitamin A and E levels were measured by HPLC.

Results:

Comparison of vitamin A and E levels between patient groups:

Vitamin A (normal reference range: 25-45 ug/dl):

In the TPN group, the mean serum vitamin A level was 26.0 $\pm$ 15 ug/dl (range: 6.1-71.4 ug/dl). In the controls, the mean serum vitamin A level was 25.0 $\pm$ 10.0 ug/dl (range: 7.4 – 66.5 ug/dl).

52% of the TPN patients had a serum vitamin A level <20 ug/dl compared to 26% in the control group ($p > 0.01$). 24% (7/29) of patients in the TPN group had vitamin A levels >2 SD above the control mean. There was no consistent trend in vitamin A levels related to duration of TPN. In both the TPN and control groups, mean serum vitamin A levels were significantly less in patients <1 yr. of age compared to those >1 yr. Mean serum vitamin A values for those <1 yr.were: on Soluvit: 22.3 $\pm$ !0.9 ug/dl; control: 20.3 $\pm$ 6.9 ug/dl. Mean serum vitamin A levels for those >1 yr. were: on Soluvit: 34.1 $\pm$ 16.0 ug/dl; control: 29.0 $\pm$ 9.5 ug/dl.

Vitamin E (normal reference range: ~0.3-1.5 mg/dl):

In the TPN group, the mean serum vitamin E level was 0.63 $\pm$ 0.24 mg/dl (range: 0.19-2.70 mg/dl). In the controls, the mean was 0.89 $\pm$ 0.31 mg/dl (range: 0.30-1.79 mg/dl). 1 patient on TPN had vitamin E levels which were >2 SD above the control mean. She was receiving a skin cream containing vitamin E. 2 patients on TPN had vitamin E levels <2 SD below the control mean. Their values were specifically 0.21 and 0.23 mg/dl. 45% of TPN patients had vitamin E levels <0.5 mg/dl (lower limit of normal for the adult) compared to 12% of controls ($p < 0.005$). (note: the ASCN recommends that vitamin E levels in full-term infants and children be maintained between 0.5-1.5 mg/dl. Levels of vitamin E <0.44 mg/dl are associated with increased hemolysis. 18% of the patients on TPN were below 0.44 mg/dl in this study).

Conclusions:

An insufficient quantity of vitamin E was administered in this study (36% of that recommended by the AMA-NAG and ASCN), resulting in inadequate blood levels of vitamin E in a number of patients. The amount of vitamin A administered in this study, exceeded AMA-NAG recommendations by ~2 fold, resulting in elevated blood levels in ~25% of the patients. No evidence of vitamin A toxicity was observed.

4. Case Report of an Allergic Reaction to Parenteral Nutrition in a Pediatric patient
Bullock et al J of parenteral and Enteral Nutrition 14(1); 98-100, 1990

A 34 month old F with neuroblastoma was receiving parenteral nutrition supplemented with MVI Pediatric. The patient experienced several episodes of hives, itching and/or rash 90 minutes-5 hrs. after starting the infusion. The episodes all promptly resolved with diphenhydramine. No allergic manifestations occurred when MVI Pediatric was removed from the TPN. Inadvertent rechallenge resulted in flushing and rash within 45 minutes of initiating the TPN infusion supplemented with MVI

Pediatric. The specific component in the MVI Pediatric responsible for these allergic reactions was not identified.

Clinical Studies to Support the use of Cernevit in Adults and Children ≥ 11 yrs.:

10 trials studying the clinical safety of Cernevit in human subjects were conducted (note: this included 1 efficacy study of 3 months duration). Cernevit was administered IM or IV injection or by IV infusion. 644 patients were entered into these trials, 593 of whom were assigned to receive Cernevit. A total of 582 patients received Cernevit. Of these 582 patients, 151 received Cernevit by the IM route, 191 via IV injection and 240 via IV infusion.

The clinical and biological safety of Cernevit was demonstrated in patients for slightly greater than 1 year (58 weeks). Efficacy was demonstrated through elimination of vitamin deficiency symptoms and the improvement of vitamins A, D and E levels in patients receiving Cernevit for home parenteral nutrition for 3 months.

Following is a summary of each of these 10 studies:

There were 2 single dose safety studies

1. Study ID: R11: Title: Single Dose Tolerance Study of Ro 12-3764/004, a RDA/NAS Based Multivitamin Supplement for Pareneteral Use With Mixed Micelles as Solubilizer.
Investigator: Dr. Valdes Quintana Study site: Argentina Date of trial: 1984

The vitamin composition of Ro 12-3764/004 (Protovit MM) is quantitatively identical to MVI-12. However, the solubilizer in Ro 12-3764/004 is a bile acid phospholipid mixed micelles.

This was an open tolerance study in 90 malnourished hospitalized patients, ages 11-80 yrs., who required a parenteral multivitamin supplement.

1 ampule of Ro 12-3764/004 was reconstituted in 2.5 ml of water for IM injection and 5 ml of water for IV injection. 1 ampule was administered daily to 90 patients as follows:

-given as an IV injection in 30 patients and IM in 30.

-added to an infusion solution (500 ml of 5% glucose or a saline solution) in 30 patients and administered over 180-360 minutes,

Local and systemic tolerance was assessed.

The only reported side effect was slight pain at the site of injection in 2 patients after IV injection (7%). In 1 of these patients the duration of the pain was <4 hrs., and, in the other patient, it lasted 12-24 hrs.

2. Study ID: R12: Title: Single Dose Tolerance Study of Ro 12-3764/004, a RDA/NAS Based Multivitamin Supplement for Pareneteral Use With Mixed Micelles as Solubilizer.
Investigator: Dr. Gonzales Study site: Uruguay

This objective and design of this study was similar to R11 above. 91 subjects: 67 malnourished patients and 24 healthy volunteers, aged 19-85 yrs., were enrolled.

1 ampule of Ro 12-3764/004 was administered as follows:

-added to an infusion solution (250-1,000 ml of dextrose, saline or a combination of both) in 30 subjects (28 patients and 2 volunteers) and administered over 130-480 minutes,

-administered as an IV injection to 30 subjects (16 patients and 14 volunteers)

-administered IM to 31 subjects (23 patients and 8 volunteers).

Local and systemic tolerance was assessed. The only reported side effect was slight pain at the site of injection in 2 subjects- one after IV (3%) (which lasted > 1 day) and 1 after IM injection (3%) (which lasted 4-12 hrs.).

There was one study of 5 days duration (safety study):

1. Study ID: R5: Title: Study of the Clinical and Biological Tolerance (Bile Acids) of the Preparation CS 021.
Investigator: Dr. Colin Study site: France Dates of study: 8/87-1/88

The vitamin composition of CS 021is similar to that of Cernevit with the following exceptions: CS 021 contains 220 IU of cholecalciferol (vitamin D) while Cernevit contains 200 IU; CS 021 contains 6 ug cyanocobalamin (B12) while Cernevit contains 5.5 ug and CS 021 contains 69 mcg of biotin while Cernevit contains 60 mcg. However, the solubilizer in CS 021 is glycocholic acid (140 mg) and lecithin (112.5 mg) is identical to that in Cernevit.

The principal aim of the study was to assess the affect of daily administration of CS 021 by IV bolus for 5 days on levels of serum bile acids and hepatic function (serum transaminasess, alkaline phosphatase and gamma GT). The investigator cites a previous study (source not stated) in healthy volunteers where a 6 day perfusion of 4.425 gms of glycocholic acid (~30 x that contained in CS 021 or in Cernevit) and 8.465 gms of lecithin (~75 x that contained in CS 021 or in Cernevit) resulted in diarrhea and abdominal cramps in all patients. The investigator postulated that this amount of glycocholic acid resulted in an oupouring of bile acids into the intestine, exceeding small intestine reabsorption capacity (the physiological biliary pool being between 1.5-3.5 gms) and leading as a result to diarrhea of colonic origin by the laxative effect of non-assimilated bile acids.

43 patients with various GI disturbances, including inflammatory bowel disease, GI malignancies, ulcers, cirrhosis, hepatitis, etc., were enrolled. Patients were to receive CS 021, 1 vial/day, by IV injection over 1 minute for 5 consecutive days. Bile acid levels were to be measured at baseline, day 4 (after the fourth injection) and day 6 (end of study). Liver function (transaminases, alkaline phosphatase, gamma GT and bilirubin), hematology and serum iron were to be measured at baseline and end of study. In addition, local and systemic tolerance was to be monitored.

Results:

There were 40 evaluable patients (reasons for exclusion of 3 patients: injections were not administered on days 3 and 5- unrelated to local or system intolerance; failure to receive the fourth and fifth injections due to transfer to oncology and IV injection from day 2 onward was not possible due to poor veins). Of these 40 patients, 17 were male and 23, female. Mean age was 50 yrs. with range of 18-85 yrs. There was no evidence of either local or systemic intolerance. There were no significant variations in the mean bile acid levels from baseline to either day 4 or day 6. Although there was no significant change in mean liver function from baseline to end of study, mean SGPT did increase from 47.5 IU/l to 60.8 IU/l ($p= 0.40$). There was no statistically significant change in either mean hematological parameters or serum iron levels. A subgroup analysis of 13 patients with inflammatory bowel disease revealed an increase in mean SGPT from baseline: 45.7 IU/l to 98.5 IU/l at the end of the study. Notable rises in SGPT occurred in 3 patients (2.3-3.4 fold increase from baseline), all of whom had elevated SGPT levels at baseline: #7- baseline was 184 IU/l and rose to 617 IU/l, #8- baseline was 93 IU/l and rose to 275 IU/l and #37- baseline was 45 IU/l and rose to 104 IU/l. Transaminase levels returned to normal within 1 week of treatment discontinuation in these 3 patients. There were no other significant changes in this subgroup analysis. The investigator concluded that administration of CS 021 to patients with inflammatory bowel disease and abnormal liver function, can exacerbate an already existing situation and recommends this preparation be avoided under such circumstances.

There were 3 studies of 5-10 days duration- all were safety studies:

1. Study ID: R8: Title: Tolerability of a Multiple Dose of Ro 12-3764/004, a Multivitamin Preparation for Parenteral Use, with Mixed Micelles as Solubilizer.
Investigator: Dr. Faintuch Study site: Brazil Date of trial : 1984

The vitamin composition of Ro 12-3764/004 (Protovit MM) is quantitatively identical to MVI-12. However, the solubilizer in Ro 12-3764/004 is a bile acid phospholipid mixed micelles.

The objective of this open, non-comparative study was to assess the local and systemic tolerability of Ro 12-3764/004 administered for 5-10 days by IV or IM injection or by infusion to hospitalized malnourished patients.

1 ampule of Ro 12-3764/004 was reconstituted in 2.5 ml of water for IM injection and 5 ml of water for IV injection. 1 ampule was administered daily as follows:

-IM injection given to 30 patients for 5 consecutive days (15 F, 15 M; mean age of 45 yrs. and range of 19-73 yrs.);
-IV injection given to 31 patients for 5 consecutive days (14 F, 17 M; mean age of 46.6 yrs. and range of 15-81 yrs.) and
-added to TPN for 10 consecutive days in 30 cases (12 F, 18

M; mean age of 44.5 yrs. and range of 15-72 yrs.) The volume of the infusion was 500-1,000 ml/day and the time of the infusion ranged from 240-720 minutes/day.

Systemic and local tolerance was assessed.

Only 2 patients (2/30= 6.6%) reported a local side effect which consisted of mild pain at the IM injection site. In 1 of these cases, the pain appeared day 2 after start of therapy and lasted <4 hrs.; in the other, it appeared on day 4 and lasted 12-24 hrs. No systemic side effects were reported.

2. Study ID: R9: Title: Tolerability of a Multiple Dose of Ro 12-3764/004, a Multivitamin Preparation for Parenteral Use, With Mixed Micelles as Solubilizer.
Investigator: Dr. Peneda Study site: Portugal Date of study: 1984

The objective of this open, non-comparative multiple dose tolerance study was to assess the local and systemic tolerability of Ro 12-3764-004 when administered for 5-10 days by IV or IM injection or by infusion.

1 ampule of Ro 12-3764/004 was reconstituted with 2.5 ml of water for IM injection and 5 ml of water for IV injection. 1 ampule was administered daily to 90 hospitalized malnourished patients as follows:

-IM injection given to 30 patients for 5 consecutive days (14 F, 16 M with mean age of 56.6 yrs. and range of 33-73 yrs.)

-IV injection given to 30 patients for 5 consecutive days (15 F, 15 M with mean age of 46.9 yrs. and range of 24-79 yrs.)

- IV infusion given to 30 patients for 10 consecutive days (7 F, 23 M with mean age of 48.1 yrs. and range of 19-77 yrs.). The infusion solution was dextrose in saline and 500 ml was infused over 180 min. in all but 1 patient in whom 1 L was administered over 360 min.

Local and systemic tolerance was assessed.

No systemic side effects occurred. Pain localized to the injection site was reported in 6/30 patients (20%) who received the drug IM. In 5 of these patients, the pain was moderate and, in 1, it was severe. The pain persisted for <4 hrs. in 4 patients, 4-12 hrs. in one and 11-24 hours in another patient.

3, Study ID: R 10: Title: Tolerability of a Multiple Dose of Ro 12-3764/004, a Multivitamin Prparation for Parenteral Use, With Mixed Micelles as Solubilizer.
Investigator: Dr. Gonzales Study site: Uruguay Date of study: 1985

The objective of this open, non-comparative multiple dose safety study was to assess the local and systemic tolerability of Ro 12-3764/004 when administered for 5-10 days by IM or IV injection or by infusion.

1 ampule of Ro 12-3764/004 was reconstituted in 2.5 ml of water for IM injection and 5 ml of water for IV injection. 1 ampule was administered daily to 86 hospitalized malnourished patients as follows:

-IM injection given to 30 patients for 5 consecutive days (8 F, 22 M with mean age of 59.3 yrs. and range of 29-80 yrs.)

- IV injection given to 30 patients for 5 consecutive days (9 F, 21 M with mean age of 61.4 yrs. and range of 28-86 yrs.)

-IV infusion given to 26 patients for 10 consecutive days (10 F, 16 M with mean age of 61.2 yrs. and range of 17-85 yrs.). The infusion solution was a combination of glucose and saline in 25 patients and was Protinutril in 1. The volume of the infusion was 1,000 ml in 25 patients and 500 ml in one. The time of the infusion was 360-480 minutes.

Local and systemic tolerance was assessed.

1 patient in the infusion group reported nausea and vomiting on study days 5, 6 and 7. The relationship of these symptoms to the test preparation was stated as "uncertain" by the investigator.

6 patients (20%) in the IM group, reported pain at the injection site, lasting <4 hrs. in two and 4-12 hrs. in the remaining patients.

4 patients (13%) in the IV injection group reported pain at the injection site. The investigator stated that this was related to the test drug in only 1 patient. In 2 of the remaining 3

patients, he attributed the local pain which occurred on the last study day, to possible irritation by the injection needle. In 1 patient, the day pain occurred was not stated.

2 patients (8%) in the infusion group reported pain at the injection site, with relation to the test drug stated as "uncertain" by the investigator. In 1 if these 2 patients, the pain was reported to be caused by infiltration.

There was 1 study of 4-57 days duration (i.e. up to 8 weeks) (safety study):

Study ID: R 13: Title: Parenteral Nutrition Supplemented with Cernevit in Patients with Liver Disease and Controls:

Investigator: Dr. Bischoff Study site: Germany Date of study: 12/95-12/96

The primary objective of this study was to determine the safety/tolerance of Cernevit in patients with liver disease receiving parenteral nutrition.

Secondary objectives included safety/tolerance of Cernevit in patients with renal insufficiency receiving parenteral nutrition and tolerance in patients with neither liver or renal insufficiency and the efficiency of vitamin supplementation by adding Cernevit to parenteral nutrition.

This was a prospective, open, controlled, comparative study in whom 64 hospitalized patients, ages 16-90 yrs., on parenteral nutrition, were recruited. Patients with and without liver disease (e.g. hepatitis, autoimmune liver disease, biliary tract disease, biliary tract disease, hepatic cancer, etc.) were assigned to receive either Cernevit or another multivitamin preparation, Soluvit/Vitalipid. The method of assignment to either Cernevit or Soluvit/Vitalipid was not described. The duration of treatment was at least 4 days for patients with liver disease and at least 7 days for those without liver disease .

The Cernevit formulation used in this study was slightly different from the currently marketed preparation in Europe with the former containing 109%-115% greater amounts of vitamins B12, biotin and D. Soluvit is quantitatively the same as M.V.I.-12 with regard to the water soluble vitamins, but, unlike M.V.I.-12, Soluvit lacks the fat soluble vitamins (A, D and E). Therefore, Soluvit was given together with Vitalipid which contains vitamins A, D and E in amounts equivalent in amounts equivalent to M.V.I.-12 (note: Vitalipid also contains vitamin K).

The following parameters were assessed:

Biochemistry and hematology profile, total bile acids, iron, transferrin, ferritin, PT, PTT, fibrinogen, factors II and V, plasma amino acid profile, vitamin B12 and folic acid: 1-2 x /week,

Serum carotene, vitamin D, PTH, immunoglobulin levels, alpha 1-antitrypsin in serum and feces and stool 24 hr. bile acids: beginning and end of study

Results:

1 patient was excluded from the analysis who received Cernevit for 4 days. Therefore, data was analyzed from 63 patients which were classified as follows:

Group 1: Patients with liver disease who received Cernevit: n= 15

Group II: Patients without liver disease who received Cernevit: n= 17

Group III: Patients with liver disease who received Soluvit/Vitalipid: n= 16

Group IV: Patients without liver disease who received Soluvit/Vitalipid: n= 15

Treatment duration for these 63 patients:

$\geq$5 - <10 days: n= 14 patients

$\geq$10-<28 days: n= 42 patients

$\geq$28 days: n= 7 patients (2 in group I, 3 in group II and 2 in group IV).

10 patients died in the study. There were 5 deaths in group I (31%). These patients had received Cernevit. The diagnoses in these 5 patients were: hepatic encephalopathy and cirrhosis; cirrhosis and hepatic failure; cirrhosis, hepatitis, GI hemorrhage and hypertension; hepatic cancer and cancer with kidney failure and pneumonia. There were 5 deaths in patients who received Soluvit/Vitalipid- 4 in group III and 1 in group IV. The diagnoses in these patients were: cirrhosis and GI hemorrhage; hepatitis and small intestine perforation; hepatic cancer; hepatic cancer, cirrhosis, pancarditis and pneumonia; and GI cancer.

Severe side effects related to study medication were not observed.

Diarrhea occurred in 6 patients: 2 patients each in groups I, II and III. No patient in any group developed icterus or pruritus during the study.

Safety parameters were compared between groups I and III and between groups I/II vs. III/IV for the following time points: week 1 (defined as the latest assessment